Inhibition of aortic aneurysm development in blotchy mice by beta adrenergic blockade independent of altered lysyl oxidase activity

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Purpose: **This study was designed to define the effects of β-adrenergic blockade on aortic lysyl oxidase (LO), an enzyme responsible for elastin and collagen cross-linking, and aneurysm formation in the blotchy mouse. It was hypothesized that β-blockade would inhibit the development of aneurysms because of its hemodynamic effect rather than a direct effect on LO activity.**
Methods: **Three groups of mice were studied: group I—normal littermates of blotchy mice; group II—untreated blotchy mice; group III—blotchy mice given either propranolol, atenolol, or nadolol. Data from the three different beta blocker–treated animals, group III, were statistically identical and were combined for analysis. The study was concluded when the mice were 4 months of age. At that time systolic blood pressure, heart rate, and aortic diameters were measured, and the entire aorta from each mouse was subjected to a bioassay for LO activity.**
Results: **Group I normal mice had an aortic arch diameter of 0.10 ± 0.02 cm. Group II blotchy mice developed aortic arch aneurysms with a diameter of 0.21 ± 0.03 cm. In Group III, β blockade reduced the aortic arch diameter in blotchy mice to 0.11 ± 0.03 cm. Mean heart rate in group III β-blocked mice was reduced 25% compared with group I normal mice, and 18% compared with group II untreated blotchy mice. Blood pressures were similar in all three groups. Group II blotchy mice exhibited approximately half of the aortic LO activity (2.43 ± 0.57 cpm/μg protein) noted in group I normal mice (5.82 ± 1.06 cpm/μg protein). Aortic LO activity in group III blotchy mice remained low (2.09 ± 0.85 cpm/μg protein) despite administration of β-blockers.**
Conclusions: **This is the first study to document an actual decrease in the level of aortic LO activity in blotchy mouse. β-Blockade inhibits development of aortic aneurysms in blotchy mice. This is associated with a reduction in heart rate, but not by alterations in LO activity.**
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Rupture of aortic aneurysms has ranked among the most common causes of death in the United States during the past decade. Surgical resection before or at time of rupture has been the only therapeutic option for these patients. However, there is evidence that pharmacologic therapy with β-adrenergic blockers can delay or prevent aneurysm formation both in experimental animal models and in human beings.[1-4] The mechanism by which β-blockade inhibits aneurysm development and rupture has been the subject of considerable speculation.

Male blotchy mice are one of the few animal models of spontaneous aortic aneurysmal disease. These mice display a phenotype consistent with a mixed connective tissue disorder that includes blotchy coat color, decreased tensile strength of skin, pulmonary emphysema, neurologic abnormalities, and aortic aneurysms.[5-8] The pathophysiologic condition of aneurysm formation in these mice has been attributed to a decrease in the activity of lysyl oxidase (LO), a copper-dependent enzyme. LO is considered

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essential in cross-linking of elastin and collagen in vascular tissue. Before this investigation, LO activity had never been assayed in aortic tissue of blotchy mice. However, reduced LO activity had been documented in lung, skin, and cultured fibroblasts of these animals.[9,10]

Propranolol has been reported by others to delay formation of aortic aneurysms in blotchy mice.[2] Furthermore, propranolol administration has been noted to increase insoluble elastin and collagen in the skin of these mice, suggesting greater matrix cross-linking.[11] On the basis of these observations, it has been believed that the delay of aneurysm formation by propranolol might be primarily due to a β-blocker–induced direct effect on LO activity and, hence, matrix cross-linking. A considerable number of assumptions would need to be true if this were to be the case.

The purpose of this study was to test the hypothesis that β-blockers retard aortic aneurysm development in the blotchy mouse by hemodynamic changes, not by altered LO activity within the aortic wall. To test this hypothesis it was necessary first to document that blotchy mouse aortic tissue indeed demonstrated decreased LO activity and that this decrease correlated with aortic aneurysm formation. Subsequently, aortic LO activity and hemodynamic responses associated with β-blocker administration in these mice were examined. Resolution of the issue of whether delayed aneurysm development accompanying β blocker administration is due to a hemodynamic effect or direct effect on LO activity is of importance in the clinical setting.

MATERIAL AND METHODS

Animal model. Male blotchy mice were bred by mating heterozygous female carriers of the blotchy gene with normal C57BL6 males. Homozygous blotchy males, distinguished by their coat color and size, and normal male littermates were weaned at 1 month of age. Animal care complied with the "Principles of Laboratory Animal Care" (National Society for Medical Research), the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23, revised 1985), and The University of Michigan Committee on the Use and Care of Animals (Approval No. 4239A Mice).

Experimental groups. Three groups of mice were studied: untreated normal male littermates of blotchy mice (group I) and untreated blotchy mice (group II) served as normal controls and blotchy controls, respectively. Additional blotchy mice were treated for 3 months with β-blockers supplied in their drinking water once they were weaned, using propranolol 0.05%, atenolol 0.05%, or nadolol 0.05% (group III).

Hemodynamic and aortic measurements. Tail cuff systolic blood pressure (mm Hg) and heart rate (beats/min) were measured in mice at the termination of the experiment when the mice were 4 months of age by use of an electrosphygmomanometer (Narco Bio-System, Houston, Texas). At that time each mouse was placed in a heated (37° C) restraining device with the tail inserted into a pneumatic cuff and a pressure transducer applied to its ventral surface. Heart rate was determined by counting pulsewave deflections on the pressure recording. A photograph was then taken of the ascending aorta, aortic arch, and descending thoracic aorta before any blood loss. A ruby bead of known diameter was used for reference to calculate the aortic diameter. The dimensions of the ascending aorta just distal to the aortic valve and the descending aorta just beyond the crossover of the azygous vein were recorded. The animals were killed, and the aorta was then removed from the aortic valve to the iliac bifurcation. This tissue in its entirety was subjected to an analysis of LO activity.

Aortic preparation. Individual mouse aortic extracts were prepared by first homogenizing the aortic tissue for 90 seconds in 0.5 ml of phosphate-buffered saline solution, 0.01 mol/L NaH_2PO_4/0.15 mol/L NaCl, pH 7.7 (NaCl/Pi). The homogenate was centrifuged (10,000 *g*, 20 minutes), and the pellet was again extracted in NaCl/Pi and centrifuged. The pellet was again extracted and centrifuged three more times for 1 hour with 0.5 ml of 4 mol/L urea/0.05 mol/L Tris, pH 7.7. All extractions were performed at 4° C. The supernatant from the three urea extracts were pooled and dialyzed against 2 L of NaCl/Pi in dialysis tubing (12,000 molecular weight cut off) for 2 hours. After dialysis, 100 μl of each LO extract were removed for determination of total protein content with a BCA protein assay reagent (Pierce, Rockford, Ill.).

Lysyl oxidase assays. LO activity was measured with a tritium release assay. Tritium release was quantitated after oxidative deamination of ^{3}H-lysine residues in labeled chick embryo elastin on incubation with LO extracted from individual mouse aortas as originally described by Pinnell and Martin[12] with modifications.[13] Briefly, each individual mouse aortic extract was divided into six microcentrifuge tubes. These tubes contained 200 μl of LO extract and 100 μl of radioactivity labeled (^{3}H-lysine) elastin substrate (approximately 100,000 counts per minute

[cpm], see details below). Three of these tubes had 100 μl of β-aminopropionitrile (BAPN 50 μg/ml), a specific inhibitor of LO. Each tube was then flushed with oxygen, vortexed, and incubated in a shaker at 37° C for 20 hours. The reaction was stopped at 20 hours by placing the tubes on ice and centrifugation (15,000 grams, 10 minutes). Tritiated water was microdistilled directly into cooled scintillation vials by the method of Misiorowski,[14] with minor modifications. The tritiated water collected in each scintillation vial was then counted in a β-scintillation counter (Beckman LS6000LL, Fullerton, Calif.).

LO activity in the extracts was calculated as the amount of tritium released from the incubation mixture of mouse aortic extract and chick elastin substrate. LO activity was reported as cpm. In the case of each extract, cpm from the three samples without BAPN were averaged as one group. The cpm from the three samples with BAPN were averaged into another group to calculate nonspecific tritium release. The latter was used as a background correction. Each averaged sample was normalized by correcting to the amount of protein in the 200 μl sample of LO extract. LO activity for each aortic sample was then calculated by subtracting the radioactivity of samples with BAPN from the radioactivity of samples without BAPN. Thus LO activity is reported as cpm per μg of aortic protein extracted.

Preparation of the elastin substrate used in the LO activity assays deserves special note. Thirty 17-day-old, chick embryo aortas were removed and preincubated for 1 hour at 37° C in 50 ml flasks containing sterile Dulbecco's modified essential medium (Gibco, Grand Island, N.Y.) without lysine but supplemented with 50 mg/L alanine, 50 mg/L glycine, 50 mg/L proline, 50 mg/L valine, 50 mg/L ascorbic acid, 10^5 units/L penicillin, 100 mg/L streptomycin, and 50 mg/L BAPN to inhibit endogenous LO activity in the substrate. The media pH was adjusted to 7.4. The chick aortas were then incubated on an orbital shaker for 20 hours in 50 ml of the above medium containing 0.56 MBq/ml (4,5-^{3}H) lysine (New England Nuclear, Wilmington, Del.) at 37° C in an atmosphere of 5% CO_2/95% air. The chick aortas were homogenized in 12 ml 0.15 mol/L NaCl and centrifuged (10,000 gm, 5 minutes) at 4° C. The pellet was retained, and this extraction step was repeated. To inactivate endogenous LO, the pellet was further homogenized in 1 N HCl and centrifuged as above.[15] This step was repeated a second time. The pellet was then homogenized in buffer (0.1 mol/L $Na_2B_4O_7$, 0.15 mol/L NaCl, pH 8.0) and centrifuged as above. This step was repeated a second time. Finally, the pellet was resuspended in assay buffer to give a concentration of 100,000 cpm per 100 μl of substrate preparation. Aliquots containing approximately 600,000 cpm were dispensed into microcentrifuge tubes and stored at −70° C for later assays. Chick elastin substrate was not stored for longer than 60 days before its use in the LO activity assay.

Statistical analysis. All data in this report are expressed as the mean ± 1 SEM. No statistically significant differences were identified among mice receiving the three different β-blockers, and thus data from these mice were combined into group III for further analysis. Group differences were subjected to statistical analysis by use of analysis of variance (ANOVA), and a post hoc comparison with Fisher's protected least squares difference test (Statview 512, Abacus Concepts, Inc., Calabasas, Calif.). Significance was assigned when the F-test result for multicomparison was $p \leq 0.05$.

RESULTS

Aortic dimensions. Group II blotchy mice all developed aortic arch aneurysms by 4 months of age (mean diameter 0.21 ± 0.03 cm) when compared with group I normal littermates (mean diameter 0.10 ± 0.01 cm, $p = 0.0001$), (Fig. 1). Group III blotchy mice treated with β-blockers exhibited a significant 47% reduction ($p = 0.0001$) in their aortic diameter (0.11 ± 0.01 cm) compared with group II untreated blotchy mice. No difference in aortic size of group III β-blocked blotchy mice existed when compared with group I normal mice.

The ratio of the ascending aortic arch diameter to the descending thoracic aorta diameter was another means of documenting arch aneurysms (Fig. 2). This ratio was 1.3 ± 0.1 in group I normal mice, whereas in group II blotchy mice it was 2.8 ± 0.5, being significantly greater ($p = 0.004$). This ratio decreased to near-normal levels of 1.6 ± 0.1 in group III blotchy mice treated with β-blockers, a size also significantly ($p = 0.004$) less than in group II mice. It is relevant that blotchy mice did not develop aneurysms in the descending thoracic aorta and that the absolute diameter of this portion of aorta did not differ among the three groups (I to III) of mice (Fig. 3).

Aortic lysyl oxidase activity. Whole aortic tissue LO activity (Fig. 4) in group II blotchy mice (2.43 ± 0.57 cpm/μg protein) was approximately half the LO activity present in aortic tissue from group I normal mice (5.82 ± 1.06 cpm/μg protein). This difference was significant ($p = 0.0025$). Aortic

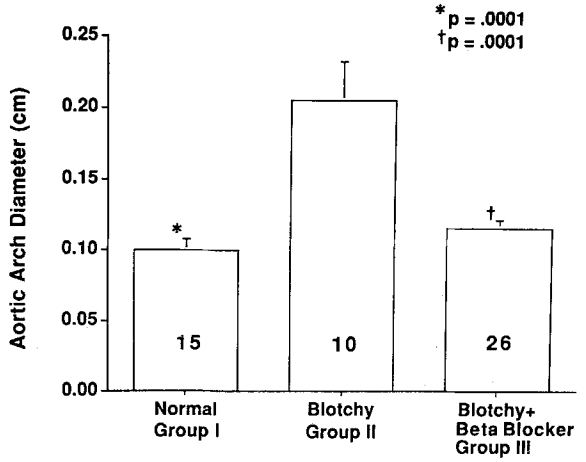

Fig. 1. Comparison of aortic arch diameters. Untreated group II-blotchy mice exhibited arch diameters (0.21 ± .03 cm) significantly greater than either group I normal littermates (0.10 ± 0.01, $p = 0.0001$) or group III blotchy mice receiving β blockers (0.11 ± 0.01 cm, $p = 0.0001$). Number within *bar* of this and all subsequent figures represents number of animals or specimens analyzed in that specific group.

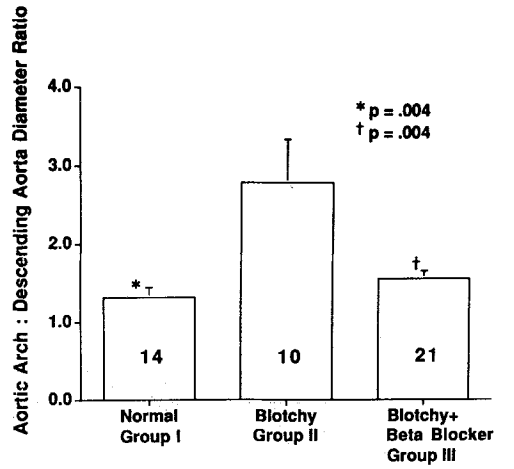

Fig. 2. Comparison of ratios of aortic arch to descending thoracic aorta diameters. Group I normal littermates ratio of 1.3 ± 0.1 was significantly less than group II blotchy mouse ratio of 2.8 ± 0.05 (p - 0.004). Group III blotchy mice receiving β blockers had ratio of 1.6 ± 0.1 that was also significantly less than group II mice ($p = 0.004$).

LO activity in group III blotchy mice receiving β-blockers (2.18 ± 0.31 cpm/μg protein) was not increased compared with untreated group II blotchy mice but was significantly less than that in group I normal mice ($p = 0.001$).

To confirm that the assay LO activity was not affected directly by propranolol, an ancillary study was performed. The entire assay as previously described was repeated with aortic tissue from group II untreated blotchy mice, but the assay incubation had propranolol directly added to it. Such an augmented assay did not result in altered LO activity measurements when compared with that without the added β-blocker (data not shown).

Hemodynamic effects. Evidence of the β-blockade was apparent in the relative bradycardia achieved in mice administered the β-blockers (Fig. 5). Heart rate averaged 699 ± 16 beats/min in group I normal mice and 638 ± 25 for group II blotchy mice. These heart rates were not statistically different. The β-blockade in group III mice produced a 25% reduction in heart rate to 521 ± 17 in comparison to group I normal mice ($p = 0.002$) and an 18% reduction compared with group II blotchy mice ($p = 0.002$). Systolic blood pressure did not differ among group I normal, group II untreated blotchy, and group III β-blocked mice (Fig. 6).

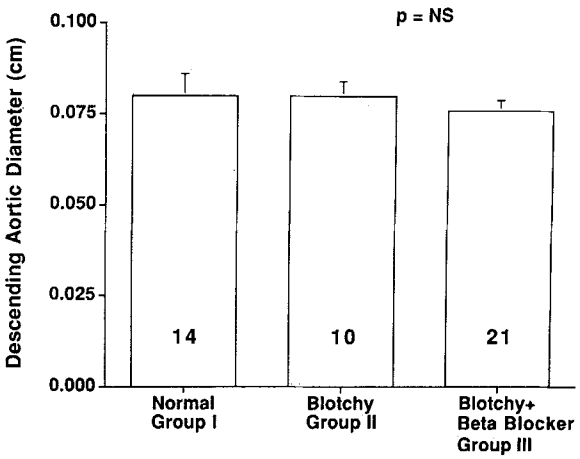

Fig. 3. Descending thoracic aortic diameters. No significant differences were detected between normal mice and blotchy mice, whether given β-blockers or not.

DISCUSSION

Mechanisms of aortic aneurysm development, enlargement, and rupture are not fully understood, but increasing evidence suggests that initial pathologic events involve matrix defects. These defects precede the inflammatory and atherogenic changes that characterize aneurysms late in their evolution.

Laboratory studies of the early events initiating aneurysmal development have been limited because of the rarity of nonprimate species spontaneously

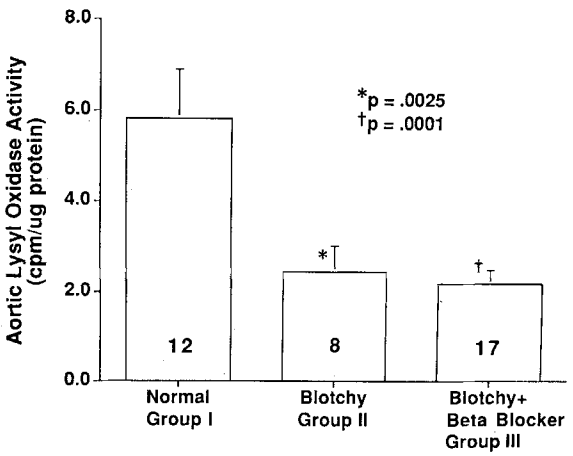

Fig. 4. Comparison of aortic LO activity. Group II blotchy mice exhibited significantly reduced LO activity (2.43 ± 0.57 cpm/μg protein) when compared with group I normal mice (5.82 ± 1.06 cpm/μg protein, $p = 0.0025$). Administration of propranolol, atenolol, or nadolol in group III did not increase aortic LO activity (2.18 ± 0.31 cpm/μg protein), but such remained significantly less than in group I normal littermates ($p = 0.0001$).

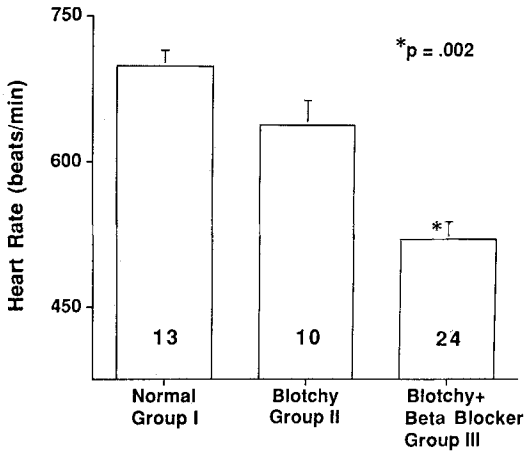

Fig. 5. Comparison of heart rates. No statistically significant difference was detected between group II blotchy (638 ± 25 beats/min) and group I normal mice (699 ± 16 beats/min). Group III blotchy mice treated with β-blockers exhibited statistically significant reduction in heart rate (521 ± 17 beats/min) compared with both blotchy and normal groups ($p = 0.002$).

exhibiting these lesions. An exception is the blotchy mouse. Its genotype contains an allelic mutation at the mottled locus of the X chromosome that results in a deficiency in a copper transport protein. This affects all proteins that require copper as a cofactor to become fully active. One of these proteins is LO, the

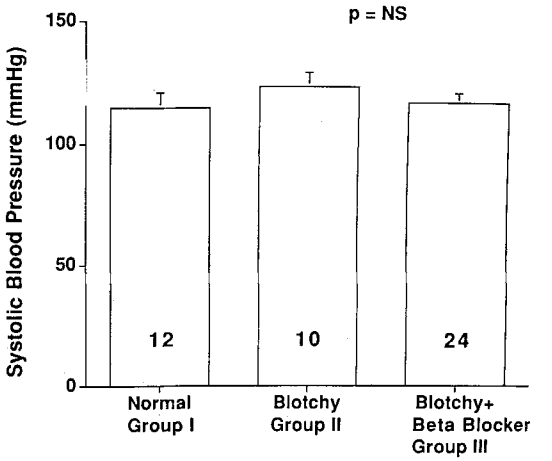

Fig. 6. Comparison of systolic blood pressure. No statistical differences could be detected between normal mice and blotchy mice, regardless of whether they were treated with β-blockers.

enzyme responsible for collagen and elastin cross-linking in the extracellular matrix of different tissue types, including the aorta.

Diminution in aortic LO activity in the genetically-impaired blotchy mouse is believed to result in inadequate cross-linking of matrix collagen and elastin. Aortic aneurysm formation occurs in 100% of blotchy mice by 6 months of age.[2,7] These aneurysms tend to affect the proximal aorta most, as evident in the present study where the blotchy mouse's aortic arch at 4 months of age was clearly aneurysmal, having a mean diameter 108% larger than their normal littermates (Fig. 1).

The 1.3:1 ratio of the aortic arch to descending thoracic aortic diameter in normal mice increased to 2.8:1 in blotchy mice, thus fulfilling the criterion of a focal aortic aneurysm. The basis for this localized aneurysmal change is not known but may relate to greater physical stresses affecting the proximal aorta as opposed to other areas of the aorta. This might necessitate greater matrix reformation, a requirement not met without normal levels of LO activity in aortic tissue. Similar stresses and impaired matrix cross-linking may account for the common infrarenal localization of human aortic aneurysms or isolated femoral and popliteal artery aneurysms occurring in male patients at sites of repetitive vessel stretch-stresses.

Decreased LO activity has been demonstrated in skin and lung of affected blotchy mice and can explain the phenotypic abnormalities seen in these tissues.[9,10] A 68% reduction in LO activity has been observed in lung tissue and appears associated with emphysema

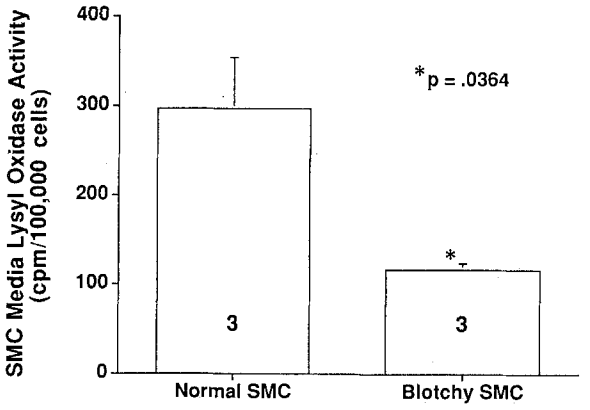

Fig. 7. Comparison of LO activity of SMC derived from normal and blotchy mouse aortas. Blotchy mouse SMC LO activity was significantly reduced to levels below that seen in normal mouse SMC ($p = 0.0364$).

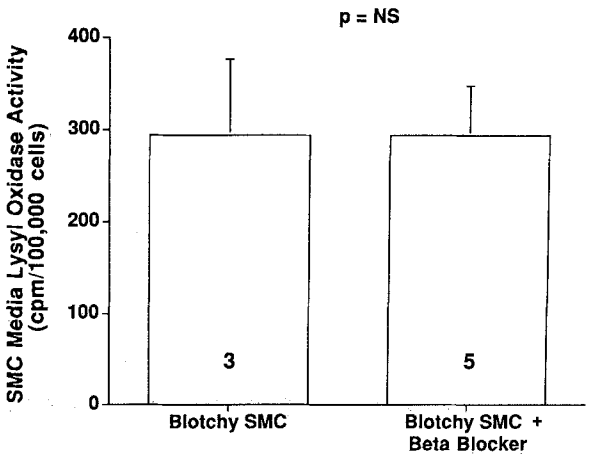

Fig. 8. Comparison of SMC derived from blotchy mice without and with addition of propranolol to culture media (repeated in triplicate). Propranolol did not alter LO activity.

in the blotchy mouse. Similarly, skin extracts in affected mice have exhibited a 69% reduction in LO activity compared with normal mice and appears associated with increased fragility of their skin. A genetically comparable human disease, Menke's syndrome, exhibits tortuosity and localized arterial dilations. Cultured fibroblasts from these patients exhibit a 74% reduction in LO activity compared with fibroblasts from normal patients.[16] Human aortic aneurysms are most likely to be infrarenal in location and when encountered clinically are associated with atherosclerotic changes. Neither of these two findings characterize blotchy mouse aneurysms. Nevertheless, the aneurysms in these mice provide a unique model to study pharmacologic interventions for arterial aneurysms in which an underlying defect exists in the vessel wall matrix.

No report to date has documented in vivo levels of aortic LO activity in blotchy mice. In addition, aortic aneurysm formation directly related to a deficiency in LO activity has never been documented. This investigation demonstrated that LO activity could be measured in individual mouse aortas and revealed a significant 59% reduction of LO activity in blotchy mouse aortas compared with normal mice. This reduction of LO activity in aortic tissue (Fig. 4) is consistent in magnitude to the reductions reported in other blotchy mouse tissues.

Decreased LO activity in blotchy aortic tissue was the subject of other in vitro experiments from our laboratory, in which cultured aortic smooth muscle cells (SMC) had propranolol (1 μmol/L) added to the culture media. SMC LO activity from blotchy mice was 61% less than that of normal SMC (Fig. 7). This reduction was almost identical to the 59% reduction of LO activity measured in whole aortic tissue. Furthermore, the addition of propranolol to blotchy SMC did not alter LO activity in the culture media (Fig. 8).

Previous investigations have documented that β-blockade with propranolol may decrease aneurysm development and lessen the frequency of aneurysm rupture. In particular, β-blockade in the blotchy mouse model has been reported to cause a 33% reduction of aortic diameter, as well as an increase in insoluble skin elastin and collagen.[2,7] The latter suggests greater matrix cross-linking. However, no direct measurements of LO activity or hemodynamic parameters were made in these studies.

In a second model involving aortic dissections in the broad-breasted white turkey, β-blockade with propranolol reduced BAPN-induced aortic rupture and raised the tensile strength of the abdominal aorta.[1,17,18] Propranolol's effect on aneurysm development was not believed to be due to the observed hemodynamic effects, a decrease in heart rate and blood pressure, but rather to be related to a direct effect on collagen and elastin matrix cross-linking through a modulation of the LO enzyme. As further support for a nonhemodynamic cause of aneurysm reduction, it was noted that hydralazine did not reduce rupture of turkey aortas, despite a reduction in blood pressure.[19] However, in as much as hydralazine did not change heart rate in these turkeys, and the fact that hydralazine is known to interfere with collagen

synthesis, the relevance of these findings is subject to question.

Propranolol has also been shown to reduce the size of experimentally induced aortic aneurysms in genetically hypertensive Wistar-Kyoto rats.[20] This effect was found to be independent of the dose and was considered to be unrelated to simple blood pressure reductions. Thus uncertainty has existed with regard to the exact mechanism by which propranolol retards development of experimental aortic aneurysms. Nevertheless, studies involving human beings with aortic aneurysms have suggested a significant reduction in aneurysmal expansion rate when patients are given β-blockers.[3,4]

Prior experiments with blotchy mice have not assessed hemodynamic alterations accompanying β-blocker reductions in aneurysm development. In this study a marked reduction in heart rate was seen in β-blocked blotchy mice, being 178 beats/min less than in normal littermates and 117 beats/min less than in untreated blotchy mice. No change in systolic blood pressure occurred with the β-blockade used in this study. The two nonselective β-blockers administered, propranolol and nadolol, produced the same reduction in heart rate, as did the cardioselective β-blocker, atenolol. Because these three β-blockers have differing chemical and structural properties, it is very unlikely that they acted by a direct mechanism on collagen or elastin cross-linking. Although heart rate and blood pressure were the only hemodynamic parameters measured in the current experiments, it is reasonable to assume that both myocardial contractility and cardiac output were also decreased. The cardiac properties of the β-blockers would appear to predominate in reducing aneurysm size because the selective β_1-antagonist was as effective as the two nonselective β-blockers.

LO alterations have not been previously hypothesized to be a significant factor in the development of human aortic aneurysms. It is certainly unlikely that a deficiency in LO activity alone is a principal cause of aortic aneurysms in human beings, although such may be a contributing factor in certain aneurysm types. The first step in aneurysm formation is believed to be a degredative process such that elastin and eventually collagen are destroyed. An abnormal increase in protelytic enzyme activity might account for such a process. However, no studies have assessed an anabolic alteration as a prime cause of aneurysm development. Adult human beings do not possess the ability to synthesize appreciable amounts of new elastin. However, collagen synthesis can be increased in response to a variety of signals. Collagen destruction can necessitate an increased demand for collagen synthesis with a concomitant need for LO activity for cross-linking if homeostasis is to be maintained. A patient with a relative deficiency in LO activity may be able to initially maintain the appropriate reparative processes to ensure usual aortic wall integrity. However, over many decades the degradative process may overwhelm the limited reparative capabilities and result in aneurysm development. Likewise, regional LO activity differences within the arterial circulation may explain certain focal sites of aneurysmal development. Documentation of LO activity levels in human arterial tissues and aortic aneurysms has not been reported.

A balance between extracellular matrix degradation and subsequent matrix synthesis exists in all aortic tissue. Thus any process that decreases matrix production or increases its degradation may result in a lessening of the aortic wall's tensile strength and increase the likelihood of aneurysm formation. If the amount of LO activity is fixed, as in the case of the blotchy mouse, with its finite ability to activate this enzyme because of a deficient cofactor, then the usual replacement of lost cross-linked collagen can not take place, and an aneurysm may develop. This may explain why the blotchy mouse develops aortic aneurysms slowly over time as opposed to being born with large aneurysms. As a corollary, if the degradative process itself is slowed by lessening the physical stresses contributing to matrix turnover, then the limited amounts of active LO may be sufficient for needed reparative processes. The reduction in hemodynamic forces accompanying the reduced heart rate in β-blocked blotchy mice may result in a decrease in the degradative phase of the normal degradation-reformation of aortic matrix. We postulate that this is the mechanism by which β-blockade results in a decrease in aneurysm development in blotchy mice.

This is the first study to measure aortic LO activity directly in the blotchy mouse. Even though blotchy mice receiving β-blockers exhibited an LO activity level that was the same as LO activity in untreated blotchy mice, they did not develop aneurysms at 4 months of age. Thus, in contrast to previous studies, data from these experiments do not support the contention that β-blockade acts directly to effect LO activity. Furthermore, it is unlikely that a direct effect of β-blockers on collagen or elastin metabolism occurs independent of LO cross-linking. The effect of β-blockade on the inhibition of aneurysm development in blotchy mice may be related to reductions in heart rate.

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DISCUSSION

Dr. Kenneth J. Cherry (Rochester, N.Y.). You have demonstrated what others have heretofore only assumed, that LO is reduced in blotchy mice with aneurysms, and you have developed assay methods that we can use. You also showed rather than assumed that propranolol has no direct effect on LO.

Previous work by others with several different models failed to show the beneficial effects of the selective β-1 drugs that you have shown, and that failure has led in part to the supposition of a direct effect of propranolol on LO and, hence, cross-linkage. Can you suggest reasons why other investigators have not been able to show the beneficial effects of the selective β-1 blockers that you have?

Because you have concluded that the reduction in heart rate is the major hemodynamic effect reducing these aneurysms, could other classes of drugs that are not antihypertensives per se, such as digoxin, be expected to have similar benefits? Should these be studied?

You described no arch or thoracic aneurysms in these treated mice. Were there abdominal aortic aneurysms present in your treated mice? Although more uncommon they have been described in blotchy mice. Would these treated mice have a normal mouse-life if they were not killed? Is this applicable to human beings? Should we be using propranolol in patients with small aneurysms, and if so in what dose?

Dr. Mohammed M. Moursi. Regarding the use of selective β-1 antagonist, we used atenolol. We are not sure why there was the same effect as in nonselective β-blockers, but certainly atenolol produced the same reduction in heart rate and the same reduction in aneurysmal size as the other β-blockers. Potentially, the other studies, the human study that used propranolol and the turkey study that used that drug, were not using a proper dose and obtaining significant hemodynamic changes such that they could have some reduction in their aneurysmal size.

Can other compounds be used that reduce heart rate? Certainly. It's important with the tools available to assess LO activity to try to investigate many other different

compounds that would decrease myocardial forces that are acting on the aneurysm.

In terms of abdominal aneurysms, these mice live approximately a year or a year and a half. Given the fact that propranolol in our hands reduced the aneurysm size to near-normal levels, we think that these animals could survive that long. We did not take any animals that far out because we wanted to have the tissue before rupture.

Is this applicable to human beings? We believe it is. The causes of aneurysms in human beings are obviously multifactorial, but in normal aortas there is a degradative-reparative process that is stable. At some point in the age of a person, you get more degradation than repair, and if you have a deficiency in some compound, maybe LO or any other compound that might cross-link or facilitate the reparative process, then potentially you shift to a more degradative process, and aneurysms develop. Anything to decrease that degradative process might be beneficial.

Dr. William H. Pearce (Chicago, Ill.). We have studied human tissue and found no difference in the elastin cross-links in patients receiving beta-blockers and those who are not. Therefore your data could be extrapolated to observation made in human beings.